THE VALUE OF THE BONE PUNCTURE FOR OBTAINING MARROW AS A DIAGNOSTIC PROCEDURE*

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EXAMINATION of the bone marrow used to be an unusual procedure during life. In many instances it was difficult to arrive at a true diagnosis in various types of anemias as well as other medical disorders, and a definite diagnosis could not be established until the bone marrow was examined at postmortem. Such patients were seen without a clear diagnosis not only during a short sojourn at the hospital, but during many years of observation.

Very little attention was paid to the suggestions of two Italian observers, namely Pianese in 1905[1] and Ghedini in 1908,[2] that marrow could be obtained during life, by trephining the femur or by the insertion of a stout trocar and canula into the tibia. In our experience, this procedure proved to be almost a major operation. This limited the number of cases in which it could advantageously be performed.

A more practical method was the trepanation of the sternum, as reported by Seyfarth in 1923.[3] This surgical procedure could be performed under local anesthesia. By this means not only could good smears be obtained from the marrow which had been scooped out from the marrow cavity, but also sections could be made for the study of the structure and the distribution of the cells such as hypoplasia or hyperplasia or their replacement by fat or fibrous tissue. A disadvantage of this method was the resulting scar over the sternum which was especially resented by female patients. Another drawback was the difficulty of repeating the biopsy and the limited number of cases that could be subjected to this procedure.

The Bone Puncture: The report of Arinkin in 1927[4] that marrow could be obtained from the sternum by means of a spinal puncture

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needle has led to a great enthusiasm for the study of the bone marrow. This procedure has many advantages:

1. It is simple.

2. It can be performed in an almost painless manner by the use of novocaine.

3. It can be used in infants and children as well as adults.

4. Repeated punctures can be performed to follow the serial changes in the bone marrow under certain conditions as reported recently in pernicious anemia by Erf and Wimer,[5] following B-12 or in multiple myeloma treated with stilbamidine by Snapper and his co-workers[6] and with urethane by Loge and Rundles.[7]

Bone marrow can be obtained in almost all cases except those in which there is an increased density of the bone (osteopetrosis), or in cases of replacement of the bone marrow by fatty, fibrous tissue or cancerous metastases. Only in this small percentage of cases is a biopsy of the sternum or of the ilium indicated.

This simple method of extracting marrow for careful study and evaluation has gained widespread use. While visiting other hospitals, I have lately been very much impressed with the increased frequency with which this procedure is employed. Since the publications from our own Hematology Laboratory by Vogel, Erf, Bassen and the author since 1937,[8-10] over 6000 additional sternal, crest or spinous process punctures were performed. Furthermore, it is noted that the communications on this subject have increased from year to year. Most of the work on this subject has been collected recently and has been well summarized, particularly in the monographs of Rohr[11] and Leitner,[12] as well as in the shorter monographs by Reich[13] and Israels.[14] These writings indicate that the bone puncture is achieving considerable importance in the study of blood diseases, especially leukemia, anemia, leukopenic states and disorders involving the blood platelets, as well as certain metabolic diseases and skeletal metastases. The results of the study of the marrow are extremely valuable for the establishment or confirmation of the underlying medical diagnosis.

Are There Any Dangers Connected With Bone Puncture? Judging from the large numbers of bone punctures performed since 1929, only a few isolated accidents have occurred. In the unusually large series of our own puncture, such as the sternum, the ilium and the spinous process, only one death has occurred. This resulted from a cardiac puncture. This

patient had already had several punctures in which, as a result of myelofibrosis, marrow could not be obtained. In only one case did a complication ensue from puncture of the spinous process, namely a partial paraplegia. This resulted from a softening of the body of the vertebra by an abscess. Only a few mishaps have been reported. Two deaths from sternal puncture have been reported by Breitenecker[15] and one each by Alder[16] and Hadorn.[17] Meyer and Halpern[18] reported a death as a result of shock and fear.

As a rule, it appears to be difficult to pierce the posterior plate of the sternum. One usually feels a "give" upon entering the marrow cavity, so that with proper precautions and increasing experience the sternal puncture can be considered a safe method.

Site of the Puncture: The term "bone puncture" is used with reference to the puncture of any bone of the skeleton for the purpose of obtaining marrow. Its cellularity varies in different bones, but the differential count is relatively unchanged, according to Nordenson.[19]

The three main areas of preference are:

1. The first, second, third bone of the sternum or manubrium.

The manubrium is preferable in infants and children as it is more cellular and accidents are less likely to occur if the posterior plate is punctured.

2. The spinous process. The use of this bone was suggested by Heidenreich and Heidenreich,[20] as well as by Loge[21] as recommended by Japanese investigators and Bickel and della Santa.[22]

3. The iliac crest. This area has been used by Nordenson,[19] van den Berghe and Blitstein.[23] In a recent report, Rubinstein[24] indicated that the iliac crest is preferable in suspected cases of multiple myeloma or skeletal metastases.

The spinous process is a good bone to use in most instances, particularly with apprehensive individuals, since the patient cannot see the procedure. The sternum should be used, if the spinous process or the crest does not yield adequate marrow.

Amount to be aspirated: The site of the puncture should be painted with iodine or some other antiseptic. The skin and periosteum should then be anesthetized with one or two per cent procaine. After a five minute wait, the needle should be inserted with a firm rotatory motion until it penetrates the bone and gives sensation of yielding. Recently we have used two syringes in the aspiration of the bone marrow. With a 20

TABLE I—DIFFERENTIAL COUNT OF NORMAL BONE MARROW

		Per Cent
Myeloblasts	—	0 — 3
Progranulocytes	—	0 — 4
Myelocytes		
Neutrophilic	—	10 — 30
Eosinophilic	—	0 — 4
Basophilic	—	0 — 1
Metamyelocytes	—	3 — 10
Band cells	—	10 — 30
Segmented		
Neutrophilic	—	10 — 30
Eosinophilic	—	0 — 4
Basophilic	—	0 — 0
Plasmacytes	—	0 — 3
Lymphocytes	—	1 — 15
Hematogones	—	0 — 10
Rubriblasts (Megaloblasts)	—	0 — 0
Prorubricytes	—	0 — 1
Rubricytes (Erythroblasts)	—	2 — 15
Metaruricytes (Normoblasts)	—	7 — 30
Average Nucleated Cells	—	100,000
Megakaryocytes	—	44

cc. syringe, we usually draw up a slight amount of blood and marrow, sufficient to cover the lower plate of the syringe. This amounts to about 0.2 cc. and is used for direct smears and a count of the nucleated cells and megakaryocytes. These smears, especially bone marrow particles, are more important for cellularity and the differential count (Table I). Immediately afterwards, with another syringe, 1 cc. of the marrow is aspirated through the same needle and injected into a small test tube containing heparin or oxalate. Some prefer the latter method, especially Reich,[13] Limarzi,[25] and the Mayo group. This method has led to the finding in the buffy coat of the characteristic cells in lupus erythematosus by Hargraves et al.[26] and in the peripheral blood by Sundberg and Lick.[27]

Microscopy of the Bone Marrow: The smears of the marrow correspond with the cell counts and present three types of cellularity, namely:

1. Hypocellular with low cell counts (5,000 to 25,000).
2. Normal cellularity (25,000 to 150,000 nucleated cells).
3. Hypercellular (150,000 to over 500,000) is normal for children.

The three main series of cells according to their frequency are the granulocytes, nucleated red cells and megakaryocytes both immature and mature. In addition, lymphocytes, hematogones and reticulum cells are present in smaller numbers. They vary considerably in the differential count as illustrated in Table I. Marked radical changes in the cellularity, in replacement or hyperplasia of certain cells are therefore important for diagnostic purposes. Attention is called to the new and improved nomenclature of the various cells in the table, especially of nucleated red cell series.[28]

Marrow Changes Occurring In Various Diseases

In a general survey of the unusually large number of bone punctures from the sternum, spinous process or iliac crest, it was found that in a restricted number of instances this procedure was of prime diagnostic importance. In others it was of confirmatory value, and in still others was chiefly of value in excluding certain suspected conditions.

A. puncture of diagnostic importance

The bone marrow proved to be diagnostic in some cases in which the peripheral blood and sometimes the clinical studies were not suggestive of any specific conditions. The most important were the following:

1. Metabolic disorders in which the bone marrow appeared relatively normal, but scattered in the smears were prominent large reticulum cells showing various types of stored lipoids.

a. In Gaucher's disease reticulum cells were characterized by the presence of needle-like kerasin crystals which rendered a reticulated appearance to the cell. In a few cases of Gaucher's disease it was possible to make this diagnosis although splenomegaly was not present.

b. Hand-Schiller-Christian's disease—the crest of the ilium is the bone of choice for finding the reticulum cells showing a diffuse vacuolization of cholesterol material.

c. Niemann-Pick's disease showed foam-like cells which contained a phosphatid substance.

II. *Protozoal Infections.* a. Kala-azar—the bone marrow shows the presence of non-lipoid reticulum cells, some of which are filled with Leishman-Donovan bodies.

b. Histoplasmosis.[29] Increase of reticulum cells showing phagocytosed histoplasma organisms.

III. *Hyperplasia of marrow cells.* The bone marrow also proves diagnostic in some cases in which the blood does not show specific changes, but the normal cells of the bone marrow are apparently displaced by a more or less marked increase of immature or abnormal cells.

a. Multiple myelomatosis. Although the diagnosis can be indicated by the presence of anemia, rouleaux formation of the red cells, hyperglobulinemia, positive formol-gel test and Bence-Jones protein in the urine, some of these changes may be absent or overlooked. An increase of plasma cells, varying from 3 to 90 per cent, definitely establishes the presence of myeloma. Multiple myelomatosis may occasionally be associated with hemolytic anemia and polycythemia, as reported recently by Lawrence and Rosenthal.[30]

b. Aleukemic leukemia. The blood picture of this form of leukemia may not show the presence of sufficient characteristic increase of immature cells in the blood. The diagnosis may be suspected by the presence of lymphocytosis, thrombocytopenia as well as lymphadenopathy or splenomegaly. A similar picture is so frequently found in other conditions (Table II) that the diagnosis can only be made with absolute certainty by the finding of a marked increase of immature cells, myeloblasts or promyelocytes or lymphoblasts in the marrow. These immature cells may appear later in the blood during the progress of the disease.

c. Follicular lymphoblastoma. The presence of a marked increase of hematogones in the marrow as well as in the blood is highly suggestive of this disease. At first sight the blood and bone marrow may resemble lymphatic leukemia. The hematogones resemble lymphocytes but have more compact and darker staining outlines, often with indented nucleus and practically no cytoplasm.[31]

IV. *Skeletal metastases.* There are some instances in which the condition may not be suspected, since the clinical picture may resemble various types of anemia, especially of the osteosclerotic type of a hemorrhagic disease. Certain x-ray findings in the bones may suggest this condition. A sternal or crest puncture may reveal either a scattered or diffuse involvement with clumps of neoplastic cells.

B. Bone Puncture of Confirmatory Value

The diagnosis of most diseases of the blood can be made by means of the history, clinical studies and especially the blood picture. The bone puncture is not the main diagnostic procedure but is helpful in confirm-

TABLE II—VARIATIONS OF THE MARROW IN LEUKOPENIC AND LYMPHOCYTIC STATES

Patient	*RBC* × 10^6	*WBC* 10^3	*Myelo-blasts*	*Myelo-cytes*	*Polynuc-lears*	*Lympho-cytes*	*Mono-cytes*	*Bone Marrow*
1.	2.95	3.2	0	3	25	71	0	Myeloblastic leukemia
2.	3.15	5.2	0	0	11	88	1	Lymphocytic leukemia
3.	4.98	5.9	0	1	53	38	5	Lymphocytic leukemia
4.	3.86	6.0	3	5	13	71	5	Progranulocytic leukemia
5.	2.38	5.3	0	1	35	53	8	Multiple myeloma
6.	5.89	3.4	0	0	45	44	10	Normal (Hypothyroid)
7.	2.94	2.1	0	0	0	94	6	Hypoplastic (Agranulocytosis)
8.	1.6	1.85	10	18	14	48	0	Aplastic (Myelofibrosis)
9.	5.4	1.0	0	0	0	69	31	Progranulocytic (Agranulocytosis)

ing the diagnosis or for repeated examinations in order to show the trend or the underlying condition. Most important of these are the following:

I. *Disorders of the leukocytic elements*, especially leukemia. From previous studies it appears that the myeloid type arises from cells in the bone marrow and may involve the lymph nodes and spleen. The cells in the marrow are most frequently the immature granulocytic elements—myeloblasts, progranulocytes, myelocytes and occasionally the plasma cells, reticulum cells and hematogones.

The second main group of leukemia arises from the lymphocytic and lymphoblastic cells in the lymphatic nodes and spleen, and usually involves the bone marrow by hyperplasia or infiltration. It is also possible that, in a few cases of lymphatic leukemia, the bone marrow may be the main center of involvement, appearing without lymphadenopathy (medullary lymphatic leukemia) or, occasionally, with only splenomegaly. It is possible that the bone marrow may not be involved at first but may become generalized at a later period.

The marrow is of greatest interest in the leukopenic states. Table II illustrates the unusually large number of blood disorders, splenic diseases

and other metabolic conditions which may show marked resemblance in the blood picture. These may show other variations, such as anemia, purpura, hepatosplenomegaly or lymphadenopathy which tend to indicate the possible underlying condition. However, the bone marrow is the most decisive factor for the differential diagnosis of leukemia, hypersplenism, Gaucher's disease, agranulocytosis (maturation arrest), hypothyroidism, constitutional or cyclic leukopenia.

II. *The anemias.* The four main groups of anemias differ very widely in the bone marrow findings. Most important are the following:

a. Macrocytic anemias, such as pernicious anemia or sprue, showing the characteristic rubriblastic (megaloblastic) marrow and showing also a tendency toward marked immaturity of the nucleated red cells.

b. Various forms of hypochromic anemia due to blood loss, neoplastic conditions, nephritis, thyroid dysfunction, either show a normal bone marrow or an increase in the rubricytes and metarubricytes (normoblasts).

c. Hemolytic anemia. The marrow usually shows marked increase of rubricytes and metarubricytes (erythroblasts and normoblasts). The aplastic phase in this condition, as reported by Owren,[32] is rare.

d. Aplastic anemia. The bone marrow consists of three types:

1. Total aplasia of the marrow including the granulocytic and megakaryocytic cells and nucleated red cell elements. The bone puncture reveals fatty and hypocellular marrow.

2. Congenital aregenerative anemia or hypoplastic anemia. The bone marrow is found to be normal except for an almost complete absence of nucleated red cells.[8]

3. Osteosclerotic anemia. The bone puncture reveals a hypoplastic marrow but the blood picture is leukemoid. This disease may be considered as a form of leukopenic myelocytic leukemia. It is also found in the late or spent phase in some cases of polycythemia. It is in these conditions that trepanation of the sternum or ilium is necessary for confirmation of this condition.

III. *Thrombocytopenia and thrombocythemia.* The marrow is of some importance in differentiating the various types of idiopathic purpura. There are three main types of myelograms according to the recent investigations in this field by Leitner,[12] Dameshek and Miller,[33] Diggs and Hewlett.[34]

a. Megakaryophthisis. Absence of giant cells in marrow is rare.

b. Variation in number of megakaryocytes from normal to very high values occurs in the main forms of chronic idiopathic purpura. In addition, there are variations in the maturation of the nuclei and cytoplasm. In general, there is a marked decrease in platelet formation from the megakaryocytes, which is corrected following splenectomy.

c. A normal megakaryocytic picture may be found in some cases of purpura accompanied often by anemia, leukopenia and splenomegaly—thrombolytic purpura—or in hypersplenism.

C. Bone Puncture for Exclusion of a Blood Condition

A majority of the punctures fall into this group. They reveal normal findings which frequently permits one to rule out a suspected hematologic condition.

This is of particular importance in certain infectious conditions such as, sepsis, infectious mononucleosis and monocytosis. The marrow is frequently examined in various leukopenic states, such as constitutional, cyclic or accompanying infections, hypothyroidism and hyperthyroidism, as well as various types of anemia to exclude some form of blood dyscrasia. It is also indicated in various types of bleeding in order to exclude an underlying cryptogenic leukemia or a genuine hemorrhagic diathesis.

In general the study of the bone marrow has now become of considerable importance not only as a diagnostic procedure as outlined above, but as an integral part of the routine procedure for a complete investigation of the patient.

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